PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
5167/478		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 94/ 04290	22/12/1994	05/08/1994
International Patent Classification (IPC) or national classification and IPC		
A61K38/17		
Applicant		
TURIANO, Angela		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consists of a total of 3 sheets.

3. This report contains indications and corresponding pages relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30/01/1996	14. 11. 96
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	I. Obrecht Telephone No. 8471

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[ ] the international application as originally filed.

[x] the description, pages 1 - 12, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____,

[x] the claims, Nos. 3, 8, 9, 10, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1, 4 - 9, 12 - 15, filed with the letter of 10.10.96,
Nos. _____, filed with the letter of _____,

[x] the drawings, sheets/fig Fig. 1 - Fig.7, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[ ] the description, pages _____.
[ ] the claims, Nos. _____.
[ ] the drawings, sheets/fig _____.

3. [x] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

Claims 2, 3, 10 and 11 relate to inhibiting replication of HIV in T4 cells, for the treatment of HIV infections or for the treatment of AIDS or ARC, in the therapy of immunodeficency syndrome. Originaly was the treatment of viral pathologies and stimulation of the immune system

as claimed in original claims 3, 9 and 10. See part V. for the details.

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 6, 7, 12 - 15	YES
	Claims 1, 4, 5, 8, 9 and originally filed 3, 8, 9, 10	NO
Inventive Step (IS)	Claims 12 - 15	YES
	Claims 1, 4 - 9 and originally filed 3, 8, 9 , 10	NO
Industrial Applicability (IA)	Claims 1, 4-9, 12-15 and originally filed 3, 8, 9, 10	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS

1). The amendments filed with the letter dated 10.10.96 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following: - a pharmaceutical composition for inhibiting replication of HIV in T4 cells (claim 2)

-for treatment of HIV infections or for the treatment of AIDS or ARC, (claim 3)

- in the therapy of immunodeficiency syndrome (in claims 10 and 11).

A basis for said amendment could not be found in the description.

Furthermore, the treatment of viral (HIV) pathologies is not supported by the description, see the description of the present application on page 11, which states that "our results showed that the contemporaneous addition of 100 ng of the substance and viral particles to the cell suspensions did not determine any inhibition of viral replication" and further, "A reduction in viral

replication was observed only following pretreatment of the cells with the AIM substance for 4 and 8 h before the HIV infection". This latter feature is anticipated by the teaching of documents (1) and (2).

Claims 2, 3, 10 and 11 are therefore not examined in their present form. The examination is carried out on claims 3, 9 and 10 as originally filed.

2). The subject-matter of the present application is concerned with a pharmaceutical composition for the treatment of cancer pathologies, characterized in that the active ingredient is consisting of major histocompatibility complex (MHC) molecules from normal tissues cells or sera, non-classical histocompatibility class 1 antigens being excluded.

Document (1) = WO-A-93 14126 discloses a major histocompatibility complex class II antigen for use as a vaccine against an immunodeficiency virus (first medical use). See in particular document (1) page 1, line 1 to 33, example 3 and the claims. The teaching of document (1) anticipates the subject-matter of claims 1, 4 to 9 since these claims are draft in form of a first medical indication and because a major histocompatibility complex class II antigen is not excluded from the scope of said claims.

Document (2) = WO-A-94 01130 discloses a major histocompatibility complex class I antigen for use as a medicament and in particular as a vaccine against an immunodeficiency virus. See in particular document (2) page 1, line 33 to page 2, line 4, example 2 and the claims. A major histocompatibility complex class I anti-

gen has been disclaimed from claims 1 and 8, and from claims 4 to 7 as far as claims 4 to 7 are dependent on claim 1, but not from claim 9. The teaching of document (2) anticipates the subject-matter of claim 9.

Under major histocompatibility complex molecules extracted from animal or human tissues, serum or cells three main classes of molecules are understood, over them: the class I, the class II and the class III.

Documents (1) and (2) does not disclose that the molecular weight is of more than 10,000 daltons, the subject-matter of claims 6 and 7 is therefore novel over the teaching of documents (1) and (2).

The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of Claims 1, 4, 5, 8 and 9 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

Document (3) = EP-A-0 563 627 discloses an anti-tumour agent comprising a non-classical histocompatibility class I antigen (first medical use form). See in particular document (3) the examples and claims 1 and 4. Non-classical histocompatibility class 1 antigens are excluded from the scope of claims 1, 4, 5, 6 and 7 but not from the scope of claim 9.
Document (3) does not disclose any specific molecular weight and that the molecular weight is of more than 10,000 daltons, the subject-matter of claims 6 and 7 is therefore novel over the teaching of document (3).

The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of Claim 9 is not new in respect of prior art as de-

fined in the regulations (Rule 64(1)-(3) PCT).

The determination of a specific molecular weight of more than 10,000 daltons is within the capabilities of the skilled man.
The present application does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of Claims 6 and 7 does not involve an inventive step (Rule 65(1)(2) PCT).

3). The subject-matter of originally filed claim 3 is concerned with a pharmaceutical composition for stimulation of the immune response of an organism, characterized in that it comprises as the active ingredient MHC molecules extracted from animal or human tissues, serum or cell, for the treatment of viral pathologies. Originally filed claims 8 and 9 are concerned with histocompatibility molecules (of different origin) for use as a medicament in stimulation of the immune system and cancer therapy and for use in sequential administration as a medicament in cancer therapy and stimulation of the immune system (first medical use claims).
This subject-matter is anticipated by the teaching of documents (1), (2) and (3) since document (1) relates to a first medical use of a major histocompatibility complex class II antigen for use as a medicament, document (2) relates to a major histocompatibility complex class I antigen for use as a medicament (first medical use claim) and document (3) relates to a non-classical histocompatibility class I antigen for use as an anti-tumour agent (first medical use form).

Originally filed claim 10 is concerned with the use of histocompatibility molecules for preparation of a pharmaceutical composition for stimulation of the immune system (and treatment of cancer pathologies: not taken

into account). This subject-matter is anticipated by the teaching of both documents (1) and (2).

The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of originally filed Claims 3, 8, 9 and 10 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT) and does not involve an inventive step (Rule 65(1)(2) PCT).

4). The subject-matter of claims 12 and 13: the use of histocompatibility molecules for the manufacture of a medicament for the treatment of cancer pathologies, wherein the active ingredient is consisting of histocompatibility molecules, non-classical histocompatibility class 1 antigen being excluded is novel over the teaching of documents (1) and (2), since the medical use is different, and novel over the teaching of document (3) because the specific class 1 of MHC is excluded.
The subject-matter of claims 12 and 13 involves an inventive step since the major histocompatibility complex molecules not comprising histocompatibility class 1 antigens are obtainable at lower costs, non-toxic and surprisingly effective as medicament for the treatment of cancer and because this specific subject-matter was not suggested in documents (1), (2) or (3).

5). **Document (4) = EP-A-0 569 678** relates to an anti-tumour vaccine comprising at least one type of tumour cell into each of which at least two genes encoding MHC proteins of different haplotypes have been inserted. See in particular document (4) the examples 10 to 14 and the claims. The present application is not concerned with cells.

Document (5) = WO-A-94 13320 discloses a pharmaceutical composition comprising an isolated immunogenic MHC polypeptide, preferably the immunogenic MHC polypeptide has a sequence from a hypervariable region of MHC molecule, in particular a MHC class II molecule, in particular a MHC class II ß chain. The immunogenic MHC polypeptide has a sequence from an MHC molecule associated with an autoimmune disease, i.a. multiple sclerosis or associated with an allergic response. The claimed subject-matter is not concerned with an isolated immunogenic MHC polypeptide, in particular a MHC class II ß chain.

6). **Document (6) = Journal of Immunological methods, vol. 112, no. 1, pages 133 to 138, M. LABETA et al. : "Solubilisation effect of Nonidet P-40, Triton X-100 and CHAPS in the detection of MHC-like glycoproteins."** discloses the step of protein solubilisation and glycoprotein isolation from cells with Nonidet P-40 at a final concentration of 0.5% (w/v), and the step of centrifugation. The supernatants were loaded on chromatography columns (*Lens culinaris*-lectin-Sepharose 4B).

The subject-matter of claims 14 and 15 differs from the teaching of document (6) in the step of dialyse of the supernatant against PBS through membranes with a cutoff of at least 10 kDa. The subject-matter of claims 14 and 15 is therefore novel over the teaching of the cited documents. The step of dialyse is also not suggested in the cited documents.

CLAIMS

1. A pharmaceutical composition for the treatment of cancer pathologies, characterized in that the active ingredient is essentially consisting of major histocompatibility complex molecules from tissues cells or sera non-classical histocompatibility class 1 antigens being excluded.

~~2. A pharmaceutical composition useful for inhibiting~~ replication of HIV in T4 cells, characterized in that the active ingredient is essentially consisting of major histocompatibility complex molecules from tissues cells or sera.

3. A pharmaceutical composition, according to claim 2, useful for treatment of viral pathologies and for treatment of HIV infections or for treatment of AIDS or ARC, the prevention of said pathologies and infections being ~~excluded.~~

4. A pharmaceutical composition, according to one of the previous claims, characterized in comprising MHC molecules of different origin, in separate packaging, for successive administration of such molecules.

5. A pharmaceutical composition, according to any previous claim, wherein said molecules originate from different species.

6. A pharmaceutical composition, according to one of the previous claims, wherein said MHC molecules are obtained as an extract from animal tissues, cells or sera by the use of

AMENDED SHEET

detergents and that they have a molecular weight of more than 10,000 daltons.

7. A pharmaceutical composition, according to claim 6, wherein such tissues, cells or sera are obtained from goat, veal, shark, chicken, pig or bovine red blood cells.

8. Histocompatibility molecules for use as essential active agent in a medicament in cancer therapy, non-classical histocompatibility class 1 antigens being excluded.

9. Histocompatibility molecules of different origin, for use in sequential administration as a medicament in cancer therapy.

~~10. Histocompatibility molecules for use as a medicament~~ in the therapy of immunodeficency syndrome.

11. Use of histocompatibility molecules for the manufacture of a medicament for the therapy of ~~immunodeficiency syndrome.~~

12. Use of histocompatibility molecules for the manufacture of a medicament for the therapy of cancer pathologies, wherein the active ingredient of said medicament is essentially consisting of said histocompatibility molecules, non-classical histocompatibility class 1 antigens being excluded.

13. Use, according to claim 11 or 12, in which such histocompatibility molecules are of different origin.

14. A process for the extraction of histocompatibility molecules according to one of the previous claims, characterized in that it comprises the following steps:

AMENDED SHEET

homogenizing the original material in the presence of Nonidet P40; centrifuging the homogenate and separating the supernatant; dialyzing the supernatant against PBS through membranes with a cutoff of at least 10 kDa.

15. A process according to claim 14, wherein such homogenization is carried out until cell lysis is substantially complete, and wherein 150 to 450 ml of PBS and 0.3 to 1.8% Nonidet P40 (v/v) are used for each 100 g of original material.

PATENT COOPERATION TREATY

[redacted]

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:
Gislon, Gabriele
MARIETTI E GISLON S.R.L.
Via Larga, 16
I-20122 Milan
ITALIE

RICEVUTO
21 NOV 1996
MARIETTI e GISLON

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	14. 11. 96
Applicant's or agent's file reference: 5167/478	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 94/04290	22/12/1994	05/08/1994

Applicant

TURIANO, Angela

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	H. Senkel Telephone No.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Washington D.C. 20231
United States of America

in its capacity as elected Office

Date of mailing (day/month/year) 26 February 1996 (26.02.96)	
International application No. PCT/EP94/04290	**Applicant's or agent's file reference**
International filing date (day/month/year) 22 December 1994 (22.12.94)	**Priority date** (day/month/year) 05 August 1994 (05.08.94)
Applicant TURIANO, Angela	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

30 January 1996 (30.01.96)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Ann Bardini

Telephone No.: (41-22) 730.91.11

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/17, C07K 1/12

A1

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(71)(72) Applicant and Inventor: TURIANO, Angela [IT/IT]; Via Goldoni, 51, I-20100 Milano (IT).

(74) Agent: GISLON, Gabriele; Marietti e Gislon S.r.l., Via Larga, 16, I-20122 Milano (IT).

(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: PHARMACEUTICAL COMPOSITIONS CONTAINING EXTRACTED MHC MOLECULES, FOR STIMULATION OF THE IMMUNE SYSTEM

(57) Abstract

Allo-, iso- and xenoantigens, chosen from histocompatibility molecules obtained by extraction from homogenates of tissues or cells with Nonidet P40 or 1 N $HClO_4$, are disclosed for use as a medicament for stimulation of the immune system of man and mammals, and in particular for cancer therapy.

PHARMACEUTICAL COMPOSITIONS CONTAINING EXTRACTED MHC MOLECULES, FOR STIMULATION OF THE IMMUNE SYSTEM

Field of the invention

The present invention concerns a pharmaceutical composition containing alloantigens and/or xenoantigens, which are useful for stimulation of the immune system, particularly for cancer therapy, i.e., for treatment of cancer in man and in mammals in general. More specifically, the composition contains, as the active ingredient, major histocompatibility complex (MHC) antigens extracted from animal tissues and with the same molecular weight (between 10,000 and 50,000 daltons).

Background of the art

In the treatment of cancer, in view of the results up to now obtained with synthetic drugs and chemical and physical therapies, investigators have sought ways to develop systems to stimulate the immune response of the organism against the proliferation of cancer cells. A factor called AF2 (alpha-fetoprotein), obtained from extracts of lambs and sheep embryos, has been used in the antitumor support therapy as an analgesic and biological antiemetic (Schweiz. Rundsch. Med. Prax. 1990, 79(16): 498-502). An antitumor polypeptide obtained from macrophage-like human cells and with a molecular weight of 47,010 daltons is also known.

In the Italian patent application no. MI 93A001369 filed in the name of the present applicant, it is described how histocompatibility antigens and ubiquitine or associated molecules administered to tumor-bearing subjects stimulate a cell-mediated and/or humoral immune response that can

produce inhibition of the growth of the tumor cells and a regression of the neoplastic disease. It is known that MHC antigens (which are ubiquitously present on the cells) perform a function of vital importance for the regulation of the immune system by taking part in the mechanism that allows the immune cells to differentiate foreign antigens (non-self) from antigens of the individual (self). The antigens and more in general the histocompatibility molecules are coded by a group of genes localized in a wide region of a single chromosome.

Three main classes of genes are recognized: *class I*, which includes genes that codify for antigens and molecules responsible for rejection and transplants and destruction of altered autologous cells; *class II*, which includes genes that codify for the surface structures that interfere in the cooperation between B and T lymphocytes and macrophages; and *class III*, which includes genes that codify for some fractions of the "complement system" and the synthesis of a serum protein (protein Ss).

The MHC antigens of class I are made up of two polypeptide chains that are not covalently linked. The largest chain, responsible for allogenicity, has a molecular weight of about 43,000 daltons. The smallest chain, identified with the β-2 microglobulin, has a molecular weight of about 13,000, is not codified by the MHC system, and is always the same for each molecule of class I. The two polypeptide chains are covalently associated to an unchanging chain of about 33,000 daltons.

The MHC antigens of class II are characterized by two glycoprotein chains - one with a molecular weight of 34,000 daltons (called alpha), and the other with a molecular weight of 29,000 daltons (called beta).

The MHC antigens are usually isolated after the cells have been destroyed by sonication, treatment with liquid nitrogen, or freezing/defrosting. Once the membranes have been prepared, histocompatibility molecules are extracted by means of a variety of detergents or by proteolysis. Salts of high ionic strength are also used for the extraction of MHC antigens from vital cells, but not from cell membranes. The greatest amount of histocompatibility antigens is obtained consistently with detergents, such as Nonidet P40 (see for an example, A. Dautigny et al, *Biochimica et Biophysica Acta*, 298:783-789, 1973). In this case, it is possible to separate the various MHC specificities by electrophoresis at a pH gradient.

Summary of the invention

It has been found that the immune response of an organism affected by a neoplasm improves considerably when MHC antigens extracted from animal tissues, serum or cells of various species (xenoantigens) are administered. Such an effect is even more pronounced and long-lasting if xenoantigens of different origin are alternated.

Objects of the invention

The object of the present invention is therefore pharmaceutical compositions for stimulation of the immune system, characterized in that they have as the main active

ingredient MHC molecules extracted from animal or human tissues, serum or cells.

The term *histocompatibility molecules* herein refers to MHC antigens and all the molecules that are codified analogously to histocompatible antigens by the corresponding genetic MHC loci, as well as their associated molecules obtained by extraction, and also refers to antigens of red blood cells.

Another object of the invention is a pharmaceutical composition of the aforementioned type and characterized by having separate packages of animal tissue, serum or cell extracts containing essentially MHC molecules of different origin for successive administration of such molecules.

The term *histocompatibility molecules of different origin* herein refers to MHC molecules obtained from different organisms or species, or originating from different batches of tissues, serums or cells.

As stated above, histocompatibility molecules useful for the invention are usually obtained by extraction from animal tissues, together with their associated molecules. The histocompatibility antigens and molecules constitute the true active ingredient, whereas their associated molecules are extracted together with the antigens and presumably act as carriers for the histocompatibility molecules.

The histocompatibility antigens and molecules used for the present invention were prepared from mammalian liver tissue, usually homogenized veal or goat liver.

Brief description of the drawings

The invention will now be further described with reference to enclosed drawings, in which :

- figures 1 to 7 are graphs showing the different tumor cell proliferation in treated and non-treated rats.

Descriprion of the preferred embodiments

Example I - Extract with acids (1 N $HClO_4$)

Veal liver homogenate (5 g) is dispersed in 10 ml of distilled water, then 10 ml of 2 N $HClO_4$ are added drop wise in 20 min under stirring at 4°C. Stirring is continued for 30 min, and the mixture is centrifuged at 100,000 × *g* for 20 min at 4°C. The supernatant is dialyzed against running water and then against distilled water all night. It is concentrated by a factor of 5 with Amicon PM 10, and a threefold volume of 4 M KCl in 0.05 M phosphate buffer at pH 7.5 is added. The mixture is stirred at 4°C for 24 h and then centrifuged at 100,000 × *g* for 1 h at 4°C. The supernatant is dialyzed against phosphate-buffered saline (PBS) and centrifuged again. Finally, the extract is concentrated by ultrafiltration through a membrane with a cutoff of 10,000 daltons up to a protein concentration of 1 mg/ml.

Example II - Extraction with detergents (PBS + Nonidet P40)

Veal liver (103 g obtained from a freshly killed animal) is cut in small pieces and homogenized in a Waring blender in 260 ml of 0.14 M PBS, pH 7.2, and 0.5% (v/v) Nonidet P40. Homogenization is done at 11,400 rpm for 2

min (alternating 30 s of treatment with 30 s of rest). Protein assays done at various phases of the treatment demonstrated that cell lysis was complete after 2 min. The sample was agitated for 45 min at 4°C and then centrifuged for 90 min at 4°C in a Sorvall SS-34 rotor at 20,500 rpm (50,000 × *g*). The supernatant (about 300 ml) was then subjected to dialysis at 4°C against 4 L of 0.14 M PBS, pH 7.2, with three changes in the dialysis phase (one change every 8 h). Membranes with a cutoff of 10 kDa were used for the dialysis.

The sample thus obtained, with a volume of 310 ml, was subjected to protein assay according to the method of Lowry, using bovine serum albumin as the calibration protein. A titer of 40.8 ± 2.2 mg protein/ml of solution was obtained. An aliquot (150 ml) of the solution was diluted to 1200 ml by addition of 0.14 M PBS at pH 7.2 and then frozen. The remaining part of the sample was frozen as such.

With the procedures of examples I and II, MHC antigens and their associated molecules with a high molecular weight (from 10,000 to 50,000) were obtained. The liver extract thus obtained, designated AIM-3, was used for in vitro and in vivo tests.

For the in vivo tests, two routes of administration were used: subcutaneous and local. The daily administration to human subjects of 4 ml of the preparation obtained with example II generally gave positive results in about 4

weeks. Neutralization of the MHC proteins by antibodies against the same MHC molecules was avoided by varying every week the origin of the extract, i.e., by using extracts of different batches obtained from tissues of the same species, or of different species.

In any case, different routes of administration can be used, such as parenteral or by aerosol. The pharmaceutical composition will consequently contain vehicles and inert substances that are pharmaceutically acceptable and chosen from those known in the technique, as a function of the chosen administration route.

The invention is now described in greater detail with reference to the following examples and relevant Figs. 1-7.

In vitro experiments

Example A

Molt 4 cells were inoculated with the extract of example I. The inoculated cells produced tissue necrosis factor (TNF) in the supernatant only at 24 h after inoculation, with concentrations clearly superior to those found in untreated cells (1000 pg/ml compared to 300 pg/ml).

In vivo experimentation

Fischer inbred rats weighing 150-175 g were used. The tumor was induced by inoculation into the pleural cavity of about 250,000 Yoshida AH-130 cells. Such a dose allowed observation of tumor growth for about 18 days before the death of the animal.

Treatment with the composition of the invention, as obtained in example I, was then given in doses of 0.025

mg/kg/day in the pleura, peritoneum or subcutaneously according to the following examples.

Example B

Some of the inoculated rats were treated with the aforementioned dose of the extract of example I (but obtained from goat liver) in the pleura (Δ), peritoneum (O) or subcutaneously (Δ) from the 4th to 8th day and then from the 12th to 16th day from cell inoculation (Fig. 1). The data (mean ± SEM of 7 experiments) showed that starting from the 8th day the number of intrapleural tumor cells was significantly less in treated rats than in controls (●) treated with a physiological solution.

Example C

In this case, the extract of example I was administered, with daily treatment from the 4th to 15th day. Fig. 2 shows that the reduction in growth of tumor cells in treated rats (O) with respect to controls (●).

Example D

Two extracts were prepared according to example I from two different batches of veal liver homogenate. The first batch was administered to inoculated rats from the 6th to 14th day, and the second batch was administered from day 15 to day 21. The results showed that the tendency of tumor cells to proliferate diminished when the extract was varied (Fig. 3). The controls (upper curve) were treated with a physiological solution as in previous examples.

Example E

In this experiment, an extract obtained as in example I from goat liver was administered from day 5 to day 10, and the same type of extract but obtained from veal liver was administered from day 11 to day 16. The results showed a marked reduction in cell growth starting from day 8 in treated rats (O) with respect to controls (●) and also with respect to the growth observed in treated rats of the previous examples (Fig. 4).

Example F

In this case, NIH-RNU$^+$ rats (Fig. 5) and NIH-RNU$^-$ rats (i.e., without the thymus) (Fig. 6) were used, and the extract of example II was administered. Fig. 5 shows the behavior of treated whole rats (curve A) and corresponding controls (curve B). Fig. 6 shows that treatment with the MHC molecules of the extract obtained according to the invention did not determine a reduction in tumor cell proliferation up to day 8, since the nude rats do not have T lymphocytes. Starting from day 9 in treated rats (curve C), there was a reduction in the growth of neoplastic cells caused by the activation of tumor specific B lymphocytes, and by stimulation of other effector cells.

Although a complete explanation of the action mechanism of the active ingredient of the present invention is not given herein, it is believed to be based on the presence of MHC antigens and molecules, which are highly immunogenic. The efficacy of such molecules is the result of the activation, by MHC proteins, of anergic lymphocytes of the tumor-bearing subject. Tumor cells normally elude the attack of

T lymphocytes since the latter, although having recognized the tumor antigens, do not receive a second signal because the tumor cells are devoid of adequate co-stimulating molecules. The molecules codified by a foreign MHC bind to T lymphocyte receptors by a mechanism analogous to that which occurs in transplant rejection and set in motion a series of biochemical events that lead to the destruction of the tumor cells. In rats devoid of T lymphocytes, to which tumor cells were inoculated, the MHC proteins complete the stimulation of B lymphocytes, which - although having recognized the tumor antigen - in the absence of helper T lymphocytes would remain inactive. The results of the following experiments confirm such a hypothesis.

Example G

Some of the inoculated rats were treated with the aforementioned dose of the extract of example II. Since no appreciable growth of tumor cells was observed, the test was repeated according to the following example.

Example H

Some of the inoculated rats were treated with the previously cited dose starting from the 5th rather than the 4th day, administering only one type of extract. Controls were treated with physiological saline. The results are shown in Fig. 7.

The possible action of the extract of example II against viral agents, particularly HIV, was also investigated. The following in vitro experimentation was carried out.

Example III

The effect of the substance, added to T-lymphoblastic cell cultures contemporaneously or at 4, 8 and 24 h before HIV infection, on viral replication was evaluated. An aliquot (100 ng) of the substance was added to cell suspensions (about 800,000 cells/ml of viral suspension) of MOLT 4 and CEM cells. The cell suspensions were infected contemporaneously and after 4, 8 and 24 h of preincubation at 37°C in 5% CO_2 of the substance under examination with 100 µl of viral suspension containing 1 × 10^4 $TICD_{50}$/ml of HIV.

Evaluation of the inhibitory effect was done by comparing the replicative levels of HIV, measured by determination of the viral P24 antigen or inverse transcriptase activity in the supernatant of pretreated cells with respect to control cells infected at the same times only with the viral suspension. Determinations of viral replication were done at 3, 6, 9, 12 and 15 days from the viral infection. Our results showed that the contemporaneous addition of 100 ng of the substance and viral particles to the cell suspensions did not determine any inhibition of viral replication. A reduction in viral replication was observed only following pretreatment of the cells with the AIM substance for 4 and 8 h before the HIV infection. Such an inhibitory effect was most evident in the first replicative phases of HIV, at 3 and 6 days from the infection, with inhibition values of 60-70%. The inhibitory effect had diminished when viral replication was assayed at 9 days from infection and reached values of 30-20% at 12-15 days

from infection. Instead, no inhibitory effect was observed when the cell cultures were pretreated for 24 h before addition of the virus.

CLAIMS

1. A pharmaceutical composition for stimulation of the immune response of an organism, characterized in that it comprises as the active ingredient MHC molecules extracted from animal or human tissues, serum or cells.

2. A pharmaceutical composition, according to claim 1, for the treatment of cancer pathologies.

3. A pharmaceutical composition, according to claim 1, for the treatment of viral pathologies.

4. A pharmaceutical composition, according to one of the previous claims, characterized in comprising MHC molecules of different origin, in separate packaging, for successive administration of such molecules.

5. A pharmaceutical composition, according to claim 4, characterized in that such molecules originate from different species.

6. A pharmaceutical composition, according to one of the previous claims, characterized in that such MHC molecules are obtained as an extract from animal tissues, cells or sera by the use of detergents and that they have a molecular weight of more than 10,000 daltons.

7. A pharmaceutical composition, according to claim 6, characterized in that such tissues, cells or sera are chosen from goat, veal or pig liver and bovine red blood cells.

8. Histocompatibility molecules for use as a medicament in stimulation of the immune system and cancer therapy.

9. Histocompatibility molecules of different origin, for use in sequential administration as a medicament in cancer therapy and stimulation of the immune system.

10. The use of histocompatibility molecules for preparation of a pharmaceutical composition for stimulation of the immune system and treatment of cancer pathologies.

11. Use, according to point 10, in which such histocompatibility molecules are of different origin.

12. A process for the extraction of histocompatibility molecules according to one of the previous points, characterized in that it comprises the following steps: homogenizing the original material in the presence of Nonidet P40; centrifuging the homogenate and separating the supernatant; dialyzing the supernatant against PBS through membranes with a cutoff of at least 10 kDa.

13. A process according to claim 12, wherein such homogenization is carried out until cell lysis is substantially complete, and wherein 150 to 450 ml of PBS and 0.3 to 1.8% Nonidet P40 (v/v) are used for each 100 g of original material.

14. A method for stimulation of the immune system of an organism for the treatment of cancer pathologies, characterized in administering to the organism MHC molecules extracted from animal or human tissues, serum or cells.

15. A method according to claim 14, characterized in administering in sequence or alternately histocompatibility molecules of different origin.

16. A method according to claim 15, wherein such histocompatibility molecules are obtained from tissues, cells or sera of different species.

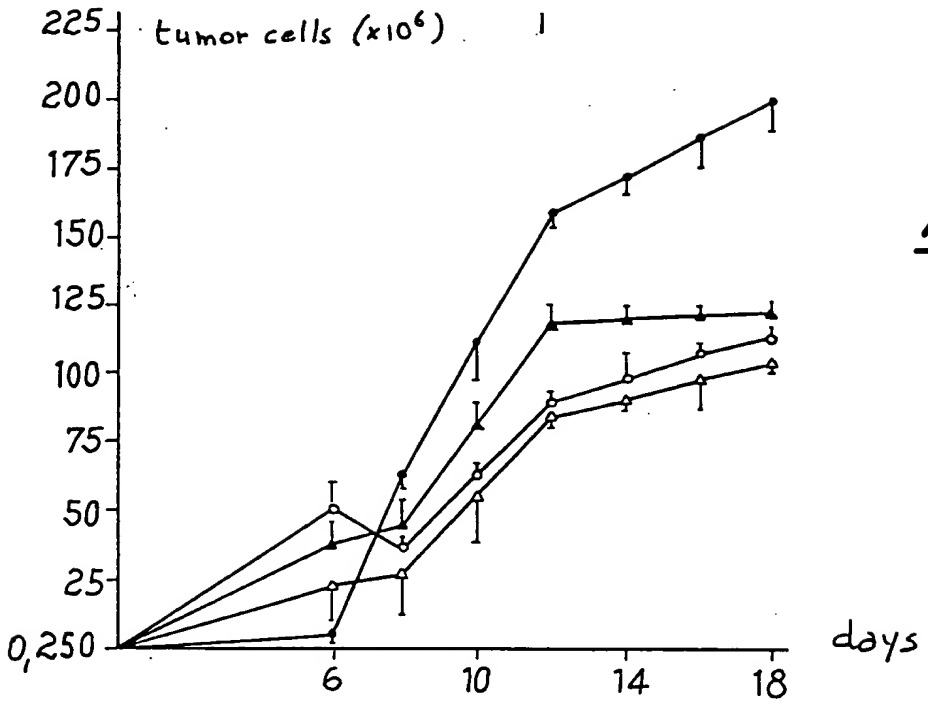

tumor cells (x10⁶)
225
200
175
150
125
100
75
50
25
0,250
6
10
14
18
days

Fig. 1

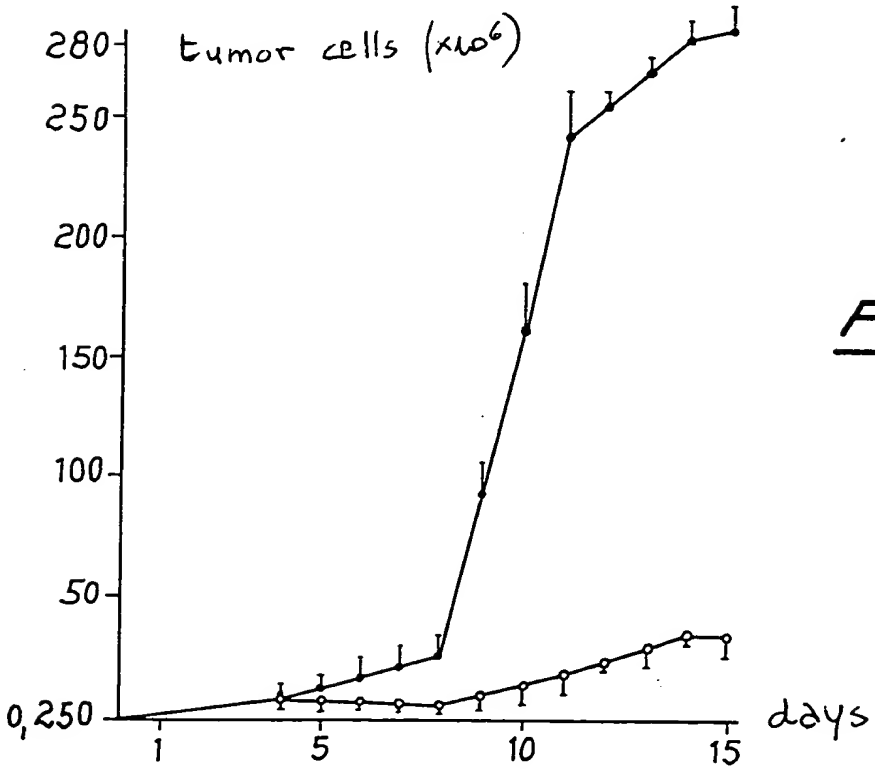

tumor cells (x10⁶)
280
250
200
150
100
50
0,250
1
5
10
15
days

Fig. 2

Fig.3

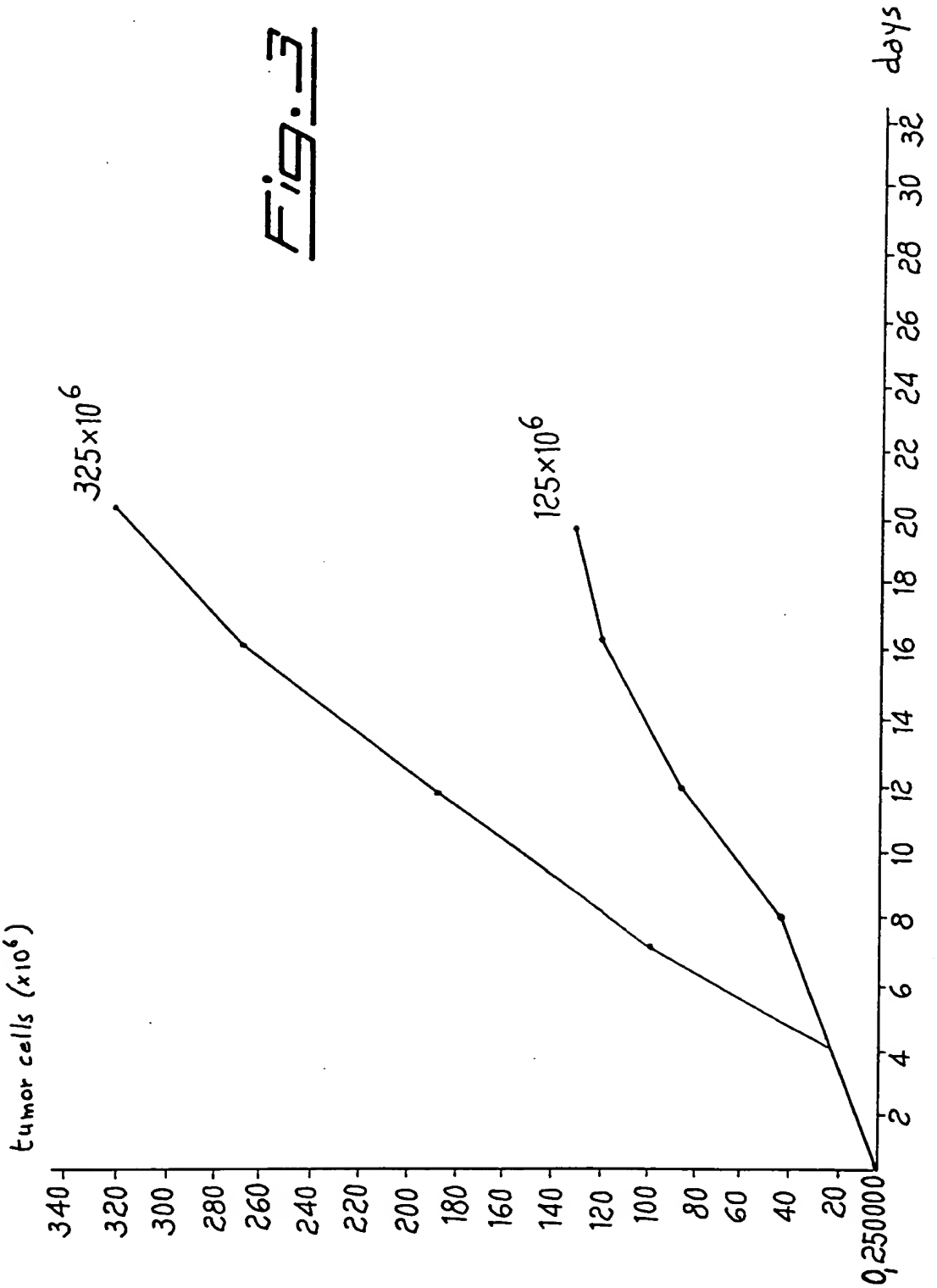
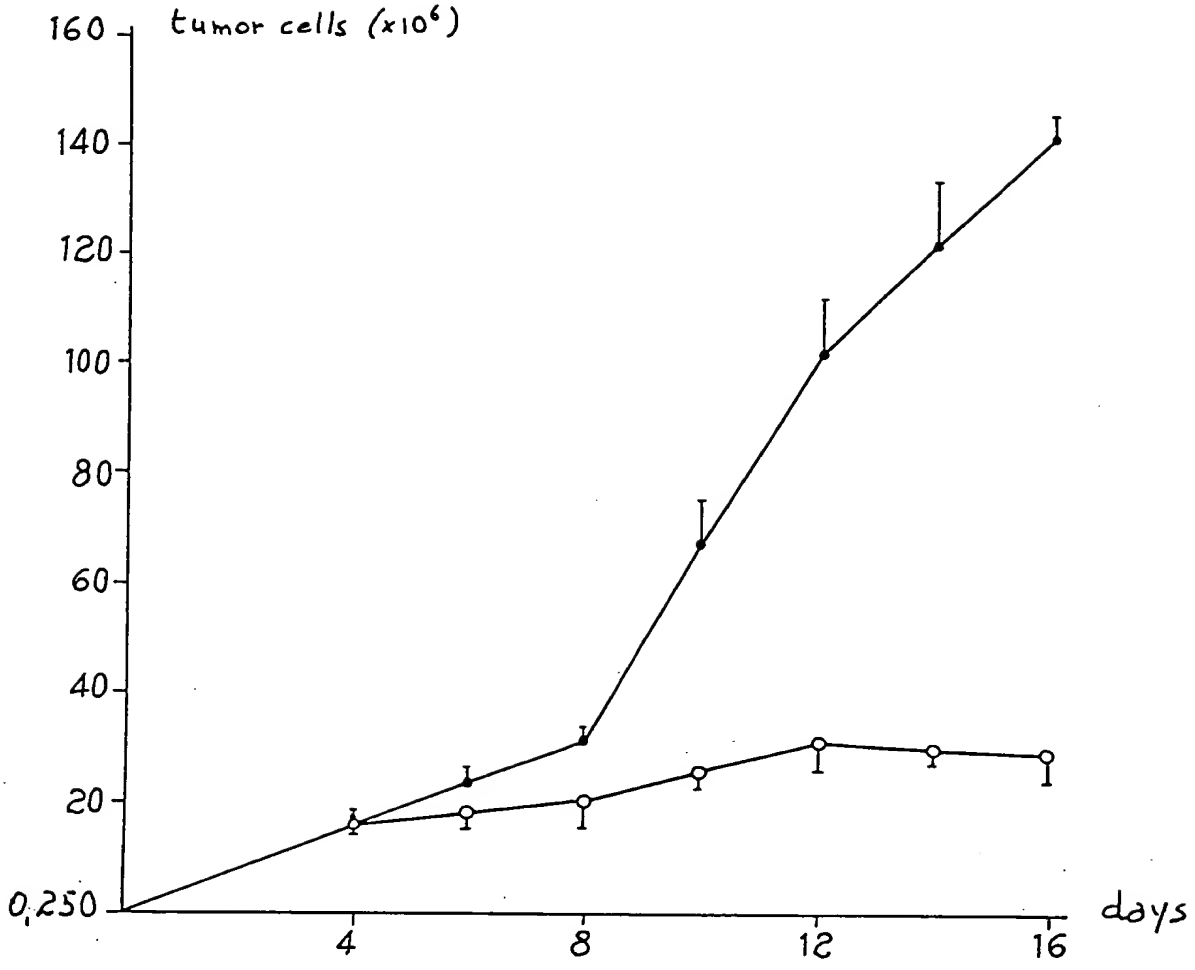

tumor cells (x10⁶)
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0,250000
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
days
325x10⁶
125x10⁶

Fig. 4



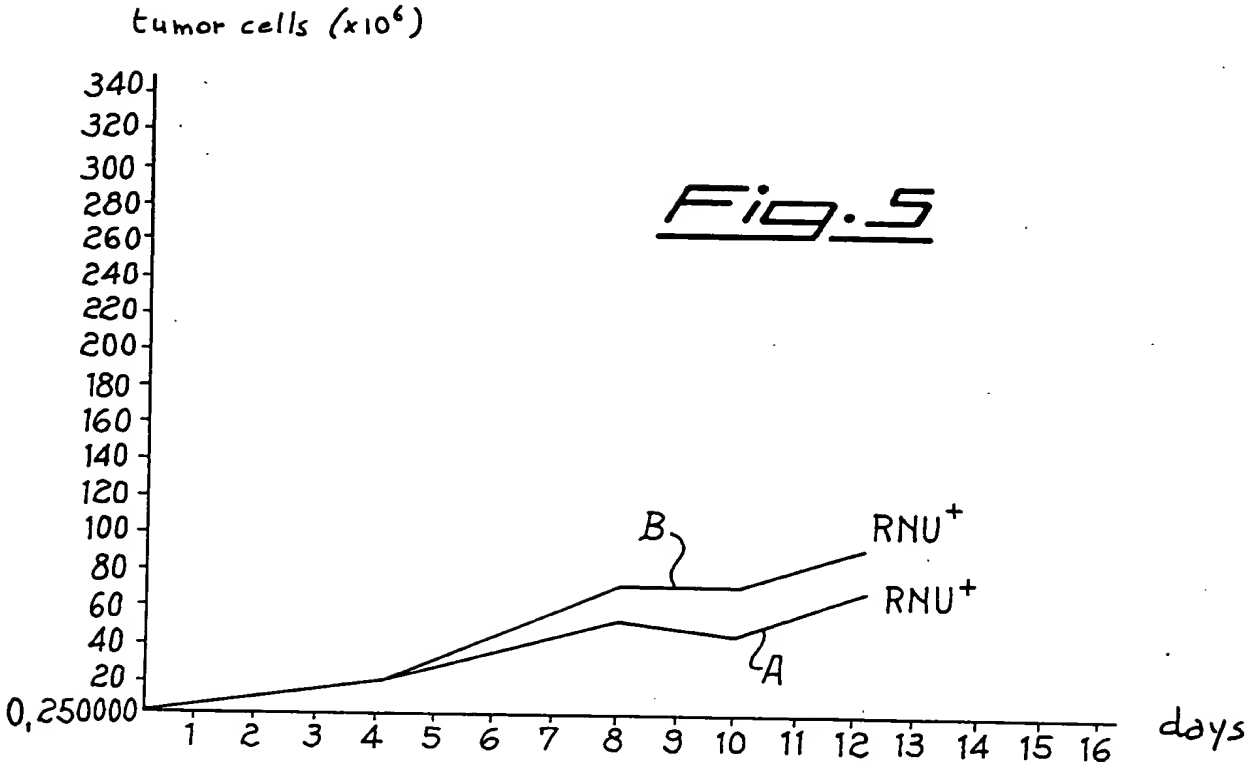

tumor cells (x10⁶)
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0,250000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
days
B
RNU+
RNU+
A

Fig. 5

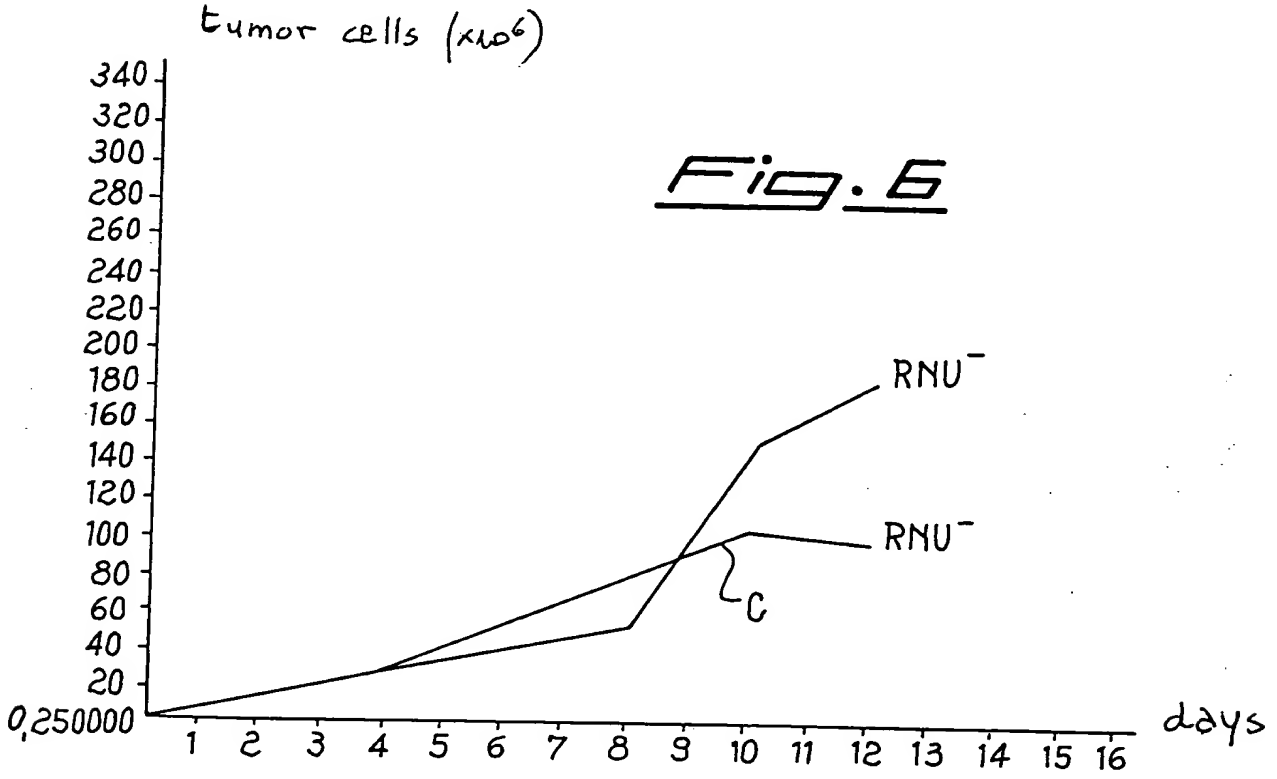
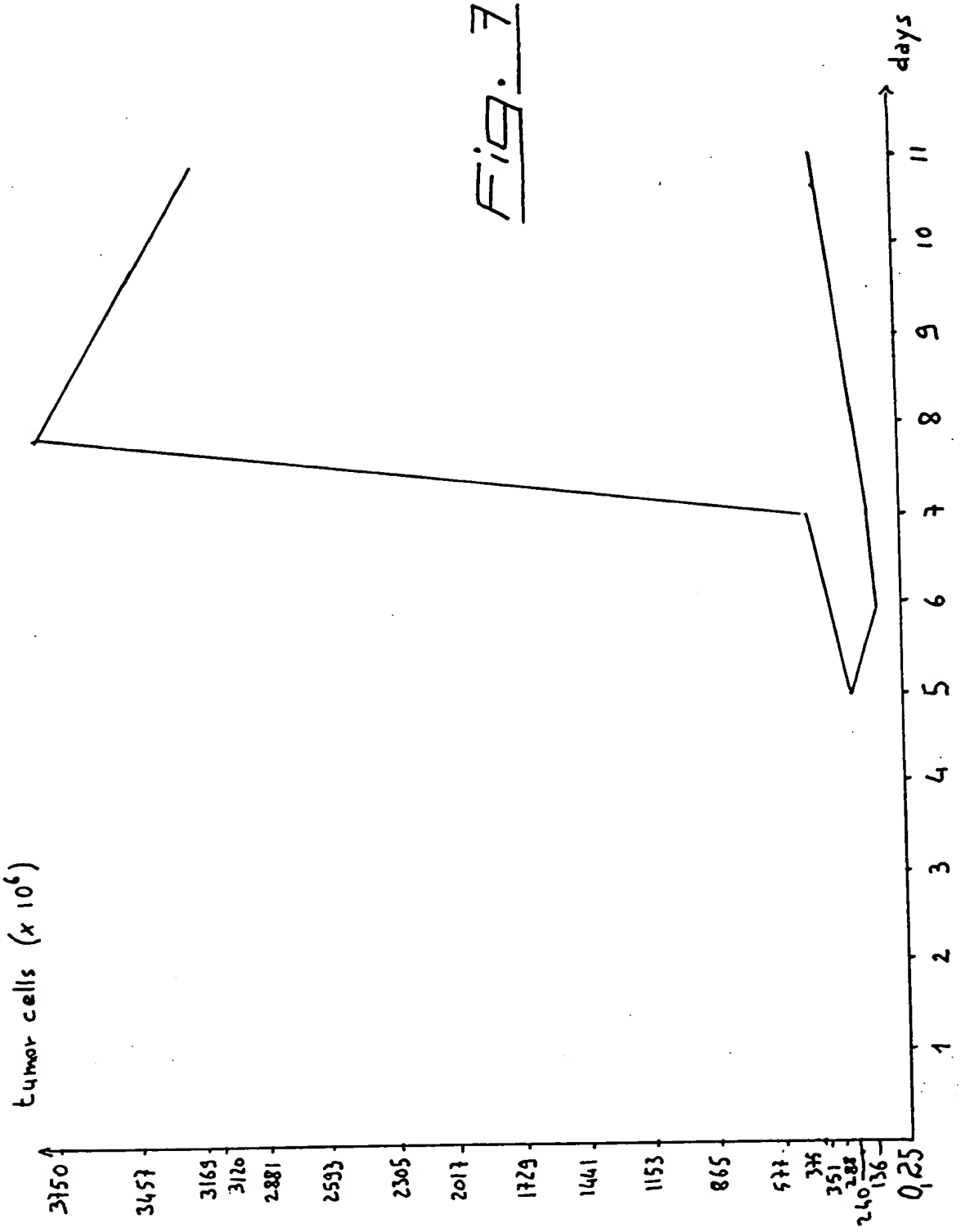

tumor cells (x10⁶)
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0,250000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
days
RNU⁻
RNU⁻
C

Fig. 6

Fig. 7


Tumor cells (x 10⁶)
3150
3457
3169
3120
2881
2593
2305
2017
1729
1441
1153
865
577
351
288
240
136
0,25
1
2
3
4
5
6
7
8
9
10
11
days

INTERNATIONAL SEARCH REPORT

I Application No
PCT/EP 94/04290

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/17 C07K1/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 14126 (MEDICAL RESEARCH COUNCIL) 22 July 1993 see page 1, line 29 - line 33 see example 3 see claims ---	8-11
X	WO,A,94 01130 (MEDICAL RESEARCH COUNCIL) 20 January 1994 see page 1, line 33 - page 2, line 4 see example 2 see claims ---	8-11
X	EP,A,0 563 627 (BIO DEFENCE INSTITUTE CO. LTD.) 6 October 1993 see examples see claims 1,4 --- -/--	8-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 1995	11 -05- 1995

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 569 678 (YEDA RESEARCH AND DEVELOPMENT CO. LTD.) 18 November 1993 see examples 10-14 see claims	8-11
X	WO,A,94 13320 (S.SRIRAM ET AL.) 23 June 1994 see examples see claims	8-11
A	JOURNAL OF IMMUNOLOGICAL METHODS, vol. 112,no. 1, 9 August 1988 AMSTERDAM, THE NETHERLANDS, pages 133-138, M. LABETA ET AL. 'Solubilisation effect of Nonidet P-40, Triton X-100 and CHAPS in the detection of MHC-like glycoproteins.' see the whole document	12,13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 94/04290

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 14-16 are directed to a method of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Noufication of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
5167/478		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 94/04290	22/12/94	05/08/94
Applicant		
TURIANO, Angela		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

   [ ] filed with the international application.

   [ ] furnished by the applicant separately from the international application,

      [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

   [ ] Transcribed by this Authority

4. With regard to the title,

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

PHARMACEUTICAL COMPOSITIONS CONTAINING EXTRACTED MHC MOLECULES, FOR STIMULATION OF THE IMMUNE SYSTEM.

5. With regard to the **abstract,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 94/04290

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 14-16 are directed to a method of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

ABSTRACT

Allo-, iso- and xenoantigens, chosen from histocompatibility molecules obtained by extraction from homogenates of tissues or cells with Nonidet P40 or 1 N $HClO_4$, are disclosed for use as a medicament for stimulation of the immune system of man and mammals, and in particular for cancer therapy.